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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/00, 15/09, 15/12		A1	(11) International Publication Number: WO 95/29233 (43) International Publication Date: 2 November 1995 (02.11.95)
(21) International Application Number: PCT/US95/04860 (22) International Filing Date: 19 April 1995 (19.04.95)		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>	
(30) Priority Data: 08/230,491 20 April 1994 (20.04.94) US			
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(54) Title: ISOLATED NUCLEIC ACID MOLECULE CODING FOR FIBROBLAST ACTIVATION PROTEIN α AND USES THEREOF			
(57) Abstract The invention describes the identification and isolation of nucleic acid molecules which code for fibroblast activation protein alpha, or "FAP α ". Various applications of the isolated molecules are also described.			

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5 **ISOLATED NUCLEIC ACID MOLECULE CODING FOR FIBROBLAST
ACTIVATION PROTEIN α AND USES THEREOF**

FIELD OF THE INVENTION

This invention relates to certain molecules associated with cancer cells and reactive with tumor stromal cells. More particularly, it relates to fibroblast activation protein alpha ("FAP α " hereafter). The molecule has previously been identified immunologically, but nucleic acid molecules coding for it had not been isolated or cloned. This, *inter alia*, is the subject of the invention. The protein has a molecular weight of from about 88 to about 95 kilodaltons as determined by SDS-PAGE. This molecule is characterized by a number of features and properties which are shared by and characteristic of membrane bound enzymes, suggesting very strongly that it, too, is a membrane bound enzyme. The nucleic acid molecules, which are a key part of the invention, are useful both as probes for cell expressing FAP α , and as starting materials for recombinant production of the protein. The recombinant protein can then be used to produce monoclonal antibodies specific for the protein, and are thus useful diagnostic agents themselves.

BACKGROUND AND PRIOR ART

The invasive growth of epithelial cancers is associated with characteristic cellular and molecular changes in the supporting stroma. For example, epithelial cancers induce the formation of tumor blood vessels, the recruitment of reactive tumor stromal fibroblasts, lymphoid and phagocytic infiltrates, the release of peptide mediators and proteolytic enzymes, and the production of an altered extracellular matrix (ECM). See, e.g., Folkman, *Adv. Cancer Res.* 43: 175-203 (1985); Basset et al., *Nature* 348: 699-704 (1990); Denekamp et al., *Cancer Metastasis Rev.* 9: 267-282 (1990); Cullen et al., *Cancer Res.* 51: 4978-4985 (1991); Dvorak et al., *Cancer Cells* 3: 77-85 (1991); Liotta et al., *Cancer Res.* 51: 5054s-5059s (1991); Garin-Chesa et al., *J. Histochem. Cytochem.* 37: 1767-1776 (1989). A highly consistent molecular trait of the stroma in several common histologic types of epithelial

5 cancers is induction of the fibroblast activation protein (FAP α), a cell surface glycoprotein with an observed M_r of 95,000 originally discovered with a monoclonal antibody, mAb F19, raised against proliferating cultured fibroblasts. See Rettig et al., Cancer Res. 46: 6406-6412 (1986); Rettig et 10 al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. USA 87: 7235-7239 (1990); Rettig et al., Cancer Res. 53: 3327-3335 (1993). Each of these four papers is incorporated by reference in its entirety.

15 Immunohistochemical studies such as those cited *supra* have shown that FAP α is transiently expressed in certain normal fetal mesenchymal tissues but that normal adult tissues are generally FAP α ⁻. Similarly, malignant epithelial, neural and hematopoietic cells are FAP α ⁻. However, most of the 20 common types of epithelial cancers, including >90% of breast, lung, skin, pancreas, and colorectal carcinomas, contain abundant FAP α ⁺ reactive stromal fibroblasts. Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990). The FAP α ⁺ tumor stromal fibroblasts almost invariably accompany 25 newly-formed tumor blood vessels, forming a distinct cellular compartment interposed between the tumor capillary endothelium and the basal aspect of malignant epithelial cell clusters. While FAP α ⁺ stromal fibroblasts are found in both primary and metastatic carcinomas, benign and premalignant epithelial 30 lesions, such as fibroadenomas of the breast and colorectal adenomas only rarely contain FAP α ⁺ stromal cells. In contrast to the stroma-specific localization of FAP α in epithelial neoplasms, FAP α is expressed in the malignant cells of a large proportion of bone and soft tissue sarcomas. Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988). Finally, 35 FAP α ⁺ fibroblasts have been detected in the granulation tissue of healing wounds (Garin-Chesa et al., *supra*). Based on the restricted distribution pattern of FAP α in normal tissues and its uniform expression in the supporting stroma of many 40 epithelial cancers, clinical trials with ¹³¹I-labeled mAb F19 have been initiated in patients with metastatic colon cancer

5 (Welt et al., Proc. Am. Assoc. Cancer Res. 33: 319 (1992)) to explore the concept of "tumor stromal targeting" for immunodetection and immunotherapy of epithelial cancers.

10 The induction of FAP α ⁺ fibroblasts at times and sites of tissue remodeling during fetal development, tissue repair, and carcinogenesis is consistent with a fundamental role for this molecule in normal fibroblast physiology. Thus, it is of interest and value to isolate and to clone nucleic acid molecules which code for this molecule. This is one aspect of the invention, which is described in detail together with other features of the invention, in the disclosure which follows.

15

BRIEF DESCRIPTION OF THE FIGURES

20 Figure 1 shows results obtained from immunoprecipitation studies carried out on detergent extracts of Trans ³⁵S-labeled cells. The study was designed to immunoprecipitate FAP α and CD26. Cell types were SW872, which is a human sarcoma cell line, COS-FAP, which is a cell line transfected with a vector coding for FAP α , i.e., pFAP-38, described in the application, and COS-CD26, which is a COS cell line transfected with a CD26 coding plasmid. Extracts were precipitated with anti-FAP α monoclonal antibody F19, anti-CD26 mAb EF-1, or a negative control mouse Ig.

25

30 Figure 2A presents Northern blot analysis of FAP α expression in a cell line (ovarian cancer SK-OV6), which has FAP α ⁻/CD26⁺ phenotype), as well as two cell lines (fibroblasts WI-38 and GM 05389), which have FAP α ⁺/CD26⁺ phenotype.

Figure 2B shows γ -actin expression for the cell lines of figure 2A.

35 Figure 3 compares the deduced amino acid sequence for FAP α , and the known sequence of CD26. The alignment has been optimized.

Figure 4 depicts heterodimer formation between FAP α and CD26 in COS-1 transfectants.

40 Figures 5A-5H, inclusive, display immunohistochemical detection of FAP α and CD26 in various cancers. In figures 5A and 5B, breast cancer is studied, for FAP α (figure 5A), and

5 CD26 (figure 5B). In figures 5C and 5D, malignant fibrous
histiocytoma are studied, for FAP α (figure 5C), and CD26
(figure 5D). Dermal scar tissue is examined in figures 5E
(FAP α), and 5F (CD26). Renal cell carcinoma is studied in
figure 5G (FAP α), and 5H (CD26).

10 DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

15 Fibroblast cell line WI-38 had been observed, previously,
to react with mAb F19 (Rettig et al., Canc. Res. 46: 6406-6412
(1986); Rettig et al., Proc. Natl. Acad. USA 85: 3110-3114
(1988); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87:
7235-7239 (1990); Rettig et al., Canc. Res. 53: 3327-3335
(1993)). It was used in the experiments which follow.

20 A cDNA library was prepared from WI-38, using well known
techniques and commercially available materials.
Specifically, the library was constructed in expression vector
pCDNA1, using the Fast Track mRNA isolation kit, and Librarium
cDNA phagemid system. Once the library was prepared, the
vectors were electroporated into cell line E. coli MC 1061/P3.
The pCDNA1 expression vector contains an antibiotic resistance
25 gene, so the E. coli were selected via antibiotic resistance.
The colonies which were resistant were then used in further
experiments. The plasmid DNA from the colonies was obtained
via alkaline lysis and purification on CsCl₂, in accordance
with Sambrook et al, Molecular Cloning: A Laboratory Manual
30 (Cold Spring Harbor Lab, Cold Spring Harbor, N.Y. 2d Ed.
1989). The technique is well known to the art, but is
incorporated by reference herein.

35 Once the plasmid DNA was isolated, it was used to
transfect COS-1 cells, which were then cultured for forty-
eight hours, after which these was tested with antibody coated
dishes. The mAbs used included F19, as described by Rettig et
al., (1986), supra, which is incorporated by reference in its
entirety. As COS-1 cells are normally FAP α ⁻, any positive
results indicated the presence of the coding sequence. The
40 immunoselection protocol was that of Aruffo et al., Proc.
Natl. Acad. Sci USA 84: 3365-3369 (1987), incorporated by

5 reference herein.

Plasmid DNA from positive clones was recovered, in accordance with Hirt, J. Mol. Biol. 26: 365-369 (1967), reintroduced into E. coli MC 1061/P3, and reselected into COS-1 cells.

10 The protocol presented herein was followed for four rounds. After this, the plasmid DNA of 50 isolated bacterial colonies was purified, using the Qiagen plasmid kit. Of the colonies, 27 clones were found to contain identical 2.8 kb inserts, as determined by EcoRI restriction enzyme mapping. 15 Several of these were found to contain FAP α -specific cDNA, via transient expression in COS-1 cells and direct immunofluorescence staining. One of these clones, i.e., "pFAP.38" was selected for further study, as elaborated upon infra.

20 Example 2

Once pFAP.38 had been identified, it was tested together with a vector coding for known cell surface marker CD26 ("pCD26"), as well as with control vector pCDNA I.

In these experiments, COS-1 cells were transfected with 25 one of pFAP.38, pCD26, or pCDNAI. After forty-eight hours, the transfectants were tested, using the well known MHA rosetting assay for cell surface antigen expression. In these experiments, mAb F19, which is FAP α specific, was used, together with mAb EF-1, which is CD26 specific. Also used 30 were four other FAP α specific mAbs, i.e., FB23, FB52, FB58 and C48. Also tested were two cancer cell lines, which are known to react with mAb F19 (SW872 liposarcoma), or EF-1 (SK-OV6 ovarian cancer). The results are set forth in Table 1, which follows.

5 **Table 1. Cell surface expression of multiple FAP α epitopes and CD26 in
human cells and COS-1 cell transfectants.**

10	Target Cell	F19	FB23	FB52	FB58	C48	EF-1
<u>Human cells</u>							
15	SW872 liposarcoma	95%	>95%	>95%	>95%	>95%	-
	SW-OV6 ovarian cancer	-	-	-	-	-	>95%
<u>COS-1 transfectants</u>							
20	COS-pCDNA1 control	-	-	-	-	-	-
	COS-pFAP 38	40%	30%	40%	20%	20%	-
25	COS-pCD26	-	-	-	-	-	40%

Example 3

Immunoprecipitation studies were then carried out to identify the antigen being targeted by the antibodies.

30 Cells were metabolically labelled with Trans 35 S-label, (ICN), extracted with lysis buffer (0.01 M Tris-HCl/0.15 M NaCl/0.01 M MgCl₂/0.5% Nonidet P-40/aprotinin (20 ug/ml)/2 mM phenylmethyl- sulfonyl fluoride), and then immunoprecipitated. The protocols used are all well known, as will be seen by reference to Rettig et al., Canc. Res. 53: 3327-3335 (1993); and Fellinger et al., Canc. Res. 51: 336-340 (1991), the disclosures of which are all incorporated by reference in their entirety. Precipitating mAbs were negative control mouse Ig, mAb F19, or EF-1. Control tests were carried out 35 with mock transfected COS-1 cells. Following immunoprecipitation, the immunoprecipitates were separated on NaDODSO₄/PAGE, under reducing conditions. In some experiments, an additional test was carried out to determine whether or not the immunoprecipitated material was 40 glycosylated. In these experiments, cell extracts were fractionated with Con A-SEPHAROSE prior to immunoprecipitation. Following immunoprecipitation, but prior 45 to fractionation on NaDODSO₄/PAGE, these precipitates were

5 digested with N-Glycanase.

The results are shown in figure 1. In COS-1 cells, pFAP.38 directs expression of an 88 kd protein species (as determined via SDS-PAGE), which is slightly smaller than the 95 kd FAP α species produced by SW872, or cultured fibroblasts. 10 Digestion with N-Glycanase produced peptides of comparable size (i.e., 74 kd versus 75 kd), showing that the glycosylation of the FAP α protein in COS-1 cells is different than in the human cell lines.

Example 4

15 Classic Northern blot analysis was then carried out, using the mRNA from FAP α^+ fibroblast cell lines WI-38 and GM 05389, and FAP α^- ovarian cancer cell line SK-OV6. Using the procedures of Sambrook et al., supra, five micrograms of mRNA from each cell line were tested. The probes used were 32 P labelled, and were prepared from a 2.3 kb ECO I fragment of pFAP.38, a 2.4 kb Hind III fragment of CD26, and a 1.8 kb BamHI fragment of γ -actin cDNA. These fragments had been purified from 1% agarose gels.

25 Figure 2 presents these results. The extracts of FAP α^+ fibroblast strains show a 2.8 kb FAP mRNA species, but extracts of SK-OV6 do not. A γ -actin mRNA species (1.8 kb), is seen in all species.

Example 5

30 The cDNA identified as coding for FAP α was subjected to more detailed analysis, starting with sequencing. The classic Sanger methodology, as set forth in Proc. Natl. Acad. Sci. USA 74: 5463-5467 (1977), was used to sequence both strands of the cDNA. Once this was secured, an amino acid sequence was deduced therefrom. This information is presented in SEQ ID 35 NO: 1. The sequence was then compared to the known amino acid sequence of CD26 (Morimoto et al., J. Immunol. 143: 3430-3437 (1989)). Figure 3 presents the comparison, using optimized sequence alignment. Any gaps in the comparison are indicated by asterisks, while identical amino acids are shown by dashes in the CD26 sequence. A hydrophobic, putative transmembrane 40 sequence is double underlined, while potential N-glycosylation

5 sites are single underlined.

The sequence analysis shows a 2812 base pair insert, wherein 2277 base pairs constitute the open reading frame. This ORF extends from start codon ATG at nucleotide 209, to stop codon TAA at 2486.

10 The deduced peptide is 759 amino acids long, and has a molecular weight of 88,233. In contrast, N-Glycanase digested, immunopurified FAP α was reported to have an estimated M_r of 75,000 on NaDODSO₄/PAGE (Rettig et al., Canc. Res. 53: 3327-3335 (1993)). A TATA box is found 83 base pairs upstream of the start codon. A polyadenylation signal and a poly-A tail were found in the 5'-untranslated region of the insert.

15 A GenBank data base search was then carried out. The most closely related genes found were those encoding dipeptidyl peptidase IV homologues (DPPIV; EC 3.4.14.5), with human DPPIV (also known as T-cell activation antigen CD26), showing 61% nucleotide sequence identity, and 48% amino acid sequence identity.

20 The second set of related genes are human, rat, and bovine homologues of DPPX, a gene of unknown function widely expressed in brain and other normal tissues. The predicted human DPPX gene product shows about 30% amino acid sequence identity with FAP α and CD26. The FAP α molecule exhibits structural features typical of type II integral membrane proteins, including a large COOH-terminal extracellular domain, a hydrophobic transmembrane segment, and a short cytoplasmic tail. The putative extracellular domain contains 25 six potential N-glycosylation sites, 13 cysteine residues (8 of which are conserved between FAP α and CD26), and three segments corresponding to highly conserved catalytic domains characteristic of serine proteases, such as DPPIV. These conserved sequences are presented in Table 2, which follows. Comparisons to DPPIV and DPPX were made via Morimoto et al., *supra*; Wada et al., Proc. Natl. Acad. Sci. USA 89: 197-201 30 (1992); Yokotani et al., Human Mol. Genet. 2: 1037-1039 (1993).

5 **Table 2. Putative catalytic domains of FAP α , DPPIV and DPPX.**

		625	701	733
	Human FAP αWGWSYEI.....	GTADDNV.....	DQNHGLS....
10	Human DPPIVWGWSYGG.....	GTADDNV.....	DEDHCIA....
	Mouse DPPIVWGWSYGG.....	GTADDNV.....	DEDHGIA....
	Rat DPPIVWGWSYGG.....	GTADDNV.....	DEDHGIA....
	Yeast DPPIVFGWSYGG.....	GTGDDNV.....	DSDHSIR....
15	Human DPPXFGKDYGG.....	PTADEKI.....	DESHYFT....
	Rat DPPXFGKDYGG.....	ATADEKI.....	DESHYFH....
	Bovine DPPXFGKDYGG.....	ATEDEKI.....	DESHYFS....

Example 6

20 An additional set of experiments were carried out to determine whether FAP α related sequences are present in non-human species. To do so, human, mouse, and Chinese hamster genomic DNA was digested using restriction enzymes, and tested, via Southern blotting, using the 2.3 kb fragment, labelled with ^{32}P , described supra. Hybridization was carried out using stringent washing conditions (0.1 x SSC, 0.1% NaDODSO₄, 68°C). Cross-hybridization was readily observed with both the mouse and hamster DNA, suggesting the existence of highly conserved FAP α homologues. In control experiments using the CD26 cDNA fragment described supra, no evidence of cross hybridization was observed.

Example 7

35 The CD26 molecule shares a number of biochemical and serological properties with FAPB, which is a previously described, FAP α associated molecule having a molecular weight of 105 kd, and found on cultured fibroblasts and melanocytes (Rettig et al., Canc. Res. 53: 3327-3335 (1993)). Cotransfection experiments were carried out to determine whether FAPB is a CD26 gene product. To test this, the same protocols were used which were used for transfection with pFAP.38 or pCD26, as described supra, but using the two

5 vectors. The results presented supra showed that cotransfection efficiency was about 40% for each vector, so about 10-20% of cell should be cotransfected.

10 Following cotransfection, the COS-1 cells were Trans ³⁵S-labeled, as described supra, then lysed, also as described supra.

15 The resulting cell extracts were separated on Con A SEPHAROSE, and the antigen (FAP α and/or CD26) were recovered in the Con A-bound fraction. The bound fraction was eluted with 0.25 M α -D-mannopyranoside. Immunoprecipitation was then carried out, as described supra, and the precipitates were separated on NaDODSO₄/PAGE, also as discussed supra.

20 Figure 4 shows these results, together with results from single transfection experiments. Those cells transfected only with pFAP.38 produce FAP α , but not FAP β (determined from mAb F19 immunoprecipitates). They also produce no CD26 antigen (tested with EF-1). Those cells transfected with pCD26 alone produce CD26 but no FAP α . Cotransfectants produce CD26 and FAP α /FAP β heteromers, as determined in the mAb F19 precipitates. This result provides direct evidence that FAP β 25 is a CD26 gene product.

Example 8

30 It has been observed previously that some cultured human cell types coexpress FAP α and CD26, and show FAP α /CD26 heteromer formation. In vivo distribution patterns of FAP α and CD26, however, as determined in previous immunohistochemical studies, appeared to be non-overlapping. 35 (See Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7329 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993); Stein et al., in Knapp et al., eds. Leukocyte typing IV-white cell differentiation antigens, pp 412-415 (Oxford University Press, N.Y. 1989), pp. 412-415; Möbius et al., J. Exp. Immunol. 74: 431-437 (1988)). In view of the potential significance of FAP α /CD26 coassociation, tissue distribution 40 was reexamined, via side by side immunohistochemical staining of normal tissues and lesional tissues known to contain FAP α .

5 fibroblasts or FAP α ⁺ malignant cells.

To test the samples, they were embedded in OCT compound, frozen in isopentane precooled in liquid nitrogen, and stored at -70°C until used. Five micrometer thick sections were cut, mounted on poly-L-lysine coated slides, air dried, and fixed in cold acetone (4°C, for 10 minutes). The sections were then tested with mAbs (10-20 ug/ml), using the well known avidin-biotin immuno-peroxidase method, as described by, e.g., Garin-Chesa et al., J. Histochem. Cytochem. 37: 1767-1776 (1989); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 15 7235-7239 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993); Garin-Chesa et al., Am. J. Pathol. 142: 557-567.

The results are shown in figure 5. Breast, colorectal, pancreas and lung carcinomas showed strong expression of FAP α and not CD26 was found (see figures 5A and 5B). Five FAP α ⁺ 20 sarcomas, including malignant fibrous histocytoma (figures 5C and 5D), were tested, and there was no expression of CD26. Examination of reactive fibroblasts of healing dermal wounds (figures 5E, 5F), showed abundant expression of both FAP α and CD26. The three renal carcinomas tested (figures 5G, 5H), 25 showed expression of CD26 in malignant epithelium. FAP α was absent from malignant epithelial cells, and showed low expression in the stroma of these carcinomas.

The foregoing examples describe an isolated nucleic acid 30 molecule which codes for fibroblast activating protein alpha ("FAP α "). The expression product of the sequence is a protein which, on SDS-PAGE, shows a molecular weight of about 75 kd. Deduced amino acid sequence, as provided in SEQ ID NO: 1, for 35 one form of the molecule, yields a molecular weight of about 88 kd. It is to be understood that, as described, FAP α may be glycosylated, with the type and amount of glycosylation varying, depending upon the type of cell expressing the molecule. The experiment described herein shows this.

The invention also comprehends the production of 40 expression vectors useful in producing the FAP α molecule. In their broadest aspect, these vectors comprise a FAP α coding sequence, operably linked to a promoter. Additional elements

5 may be a part of the expression vector, such as genes which confer antibiotic resistance, amplifiable genes, and so forth.

The coding sequences and vectors may also be used to prepare cell lines, wherein the coding sequence or expression vector is used to transfect or to transform a recipient host. 10 The type of cell used may be prokaryotic, such as E. coli, or eukaryotes, such as yeast, CHO, COS, or other cell types.

15 The identification of nucleic acid molecules such as that set forth in SEQ ID NO: 1 also enables the artisan to identify and to isolate those nucleic acid molecules which hybridize to it under stringent conditions. "Stringent condition" as used herein, refers to those parameters set forth supra, whereby both murine and hamster sequences were also identified. It will be recognized by the skilled artisan that these conditions afford a degree of stringency which can be achieved 20 using parameters which vary from those recited. Such variance is apprehended by the expression "stringent conditions".

25 The ability of nucleic acid molecules to hybridize to complementary molecules also enables the artisan to identify cells which express FAP α , via the use of a nucleic acid hybridization assay. One may use the sequences described in the invention to hybridize to complementary sequences, and thus identify them. In this way, one can target mRNA, e.g., which is present in any cell expressing the FAP α molecule.

30 It is of course understood that the nucleic acid molecules of the invention are also useful in the production of recombinant FAP α . The recombinant protein may be used, e.g., as a source of an immunogen for generation of antibodies akin to known mAb F19, and with the same uses. Similarly, the recombinant protein, and/or cells which express the molecule 35 on its surface, may be used in assays to determine antagonists, agonists, or other molecules which interact with the FAP α molecule. Such molecules may be, but are not necessarily limited to, substrates, inhibiting molecules, antibodies, and so forth. This last feature of the invention 40 should be considered in light of the observed structural resemblances to membrane bound enzymes. This type of molecule

5 is associated with certain properties which need not be described in detail here. It will suffice to say that inhibition or potentiation of these properties as associated with FAP α is a feature of this invention. For example, one may identify substrates or the substrate for the FAP α molecule, via the use of recombinant cells or recombinant FAP α per se. The substrates can be modified to improve their effect, to lessen their effect, or simply to label them with detectable signals so that they can be used, e.g., to identify cells which express FAP α . Study of the interaction of 10 substrate and FAP α , as well as that between FAP α and any molecule whatsoever, can be used to develop and/or to identify 15 agonists and antagonists of the FAP α molecule.

Other aspects of the invention will be clear to the skilled artisan, and need not be set forth here.

20 The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described 25 or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

5 (1) GENERAL INFORMATION:

(i) APPLICANTS: Rettig, Wolfgang J.; Scanlan, Matthew J.;
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10 (ii) TITLE OF INVENTION: ISOLATED NUCLEIC ACID MOLECULE CODING
FOR FIBROBLAST ACTIVATION PROTEIN α AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 1

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
25 (B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:

30 (A) APPLICATION NUMBER: 08/230,491
(B) FILING DATE: 20-APRIL-1994
(C) CLASSIFICATION: 435

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(C) REFERENCE/DOCKET NUMBER: LUD 330

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5 (2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2812 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

	AAGAACGCC	CCAAAATCTG	TTTCTAATT	TACAGAAATC	TTTGAAACT	TGGCACGGTA	60
	TTCAAAAGTC	CGTGGAAAGA	AAAAAACCTT	GTCCTGGCTT	CAGCTTCAA	CTACAAAGAC	120
15	AGACTTGGTC	CTTTCAACG	GTTTACAG	ATCCAGTGC	CCACGCTCTG	AAGACAGAA	180
	TAGCTAAC	TTCAAAACAT	CTGGAAAAAT	GAAGACTTGG	GTAAAATCG	TATTTGGAGT	240
	TGCCACCTCT	GCTGTGCTT	GCTTATTGGT	GATGTGCATT	GTCTTACGCC	CTTCAAGAGT	300
	TCATAACTCT	GAAGAAAATA	CAATGAGAGC	ACTCACACTG	AAGGATATT	TAAATGGAAC	360
	ATTTCTTAT	AAAACATT	TTCCAAACTG	GATTCAGGA	CAAGAATATC	TTCATCAATC	420
20	TGAGATAAC	AATATAGTAC	TTTATAATAT	TGAAACAGGA	CAATCATATA	CCATTTGAG	480
	TAATAGAAC	ATGAAAAGTG	TGAATGCTTC	AAATTACGGC	TTATCACCTG	ATCGGCAATT	540
	TGTATATCTA	GAAAGTGATT	ATTCAAAAGCT	TTGGAGATAC	TCTTACACAG	CAACATATTA	600
	CATCTATGAC	CTTAGCAATG	GAGAATTGT	AAGAGGAAAT	GAGCTTCCTC	GTCCAATTCA	660
	GTATTTATGC	TGGTCGCCTG	TTGGGAGTAA	ATTAGCATAT	GTCTATCAA	ACAATATCTA	720
25	TTGAAACAA	AGACCAGGAG	ATCCACCTTT	TCAAATAACA	TTAATGGAA	GAGAAAATAA	780
	AATATTTAAT	GGAATCCCAG	ACTGGGTTTA	TGAAGAGGAA	ATGCTTCCTA	CAAATATGC	840
	TCTCTGGTGG	TCTCCTAATG	GAAAATTTT	GGCATATGCG	GAATTAATG	ATAAGGATAT	900
	ACCAAGTTATT	GCCTATTCC	ATTATGGCGA	TGAACAATAT	CCTAGAACAA	TAAATATTCC	960
	ATACCCAAAG	GCTGGAGCTA	AGAATCCCGT	TGTTCGGATA	TTTATTATCG	ATACCACTTA	1020
30	CCCTGCAT	GTAGGTCCCC	AGGAAGTGCC	TGTTCCAGCA	ATGATAGCCT	CAAGTGATTA	1080
	TTATTCAGT	TGGCTCACGT	GGGTTACTGA	TGAACGAGTA	TGTTGCAGT	GGCTAAAAG	1140
	AGTCCAGAA	GTTCGGTCC	TGTCTATATG	TGACTTCAGG	GAAGACTGGC	AGACATGGGA	1200
	TTGTCCAAAG	ACCCAGGAGC	ATATAGAAGA	AAGCAGAACT	GGATGGGCTG	GTGGATTCTT	1260
	TGTTCAAGA	CCAGTTTCA	GCTATGATGC	CATTTCGTAC	TACAAAATAT	TTAGTGACAA	1320
35	GGATGGCTAC	AAACATATT	ACTATATCAA	AGACACTGTG	GAAAATGCTA	TTCAAATTAC	1380
	AAGTGGCAAG	TGGGAGGCCA	TAAATATATT	CAGAGTAACA	CAGGATTAC	TGTTTTATTC	1440
	TAGCAATGAA	TTTGAAGAAT	ACCCTGGAAG	AAGAAACATC	TACAGAATT	GCATTGGAAG	1500
	CTATCCTCCA	AGCAAGAAGT	GTGTTACTTG	CCATCTAAGG	AAAGAAAGGT	GCCAATATTA	1560
	CACAGCAAGT	TTCAGCGACT	ACGCCAAGTA	CTATGCAC	GTCTGCTACG	GCCCAGGCAT	1620
40	CCCCATTCC	ACCCTTCATG	ATGGACGCAC	TGATCAAGAA	ATTAAAATCC	TGGAAGAAAA	1680
	CAAGGAATTG	GAAAATGCTT	TGAAAAATAT	CCAGCTGCCT	AAAGAGGAAA	TTAAGAAACT	1740

5 TGAAGTAGAT GAAATTACTT TATGGTACAA GATGATTCTT CCTCCTCAAT TTGACAGATC 1800
AAAGAAGTAT CCCTTGCTAA TTCAAGTGTA TGGTGGTCCC TGCAGTCAGA GTGTAAGGTC 1860
TGTATTTGCT GTTAATTGGA TATCTTATCT TGCAAGTAAG GAAGGGATGG TCATTGCCTT 1920
GGTGGATGGT CGAGGAACAG CTTCCAAGG TGACAAACTC CTCTATGCAG TGTATCGAAA 1980
GCTGGGTGTT TATGAAGTTG AAGACCAAGAT TACAGCTGTC AGAAAATTCA TAGAAATGGG 2040
10 TTCATTGAT GAAAAAAAGAA TAGCCATATG GGGCTGGTCC TATGAGATAC GTTTCATCAC 2100
TGGCCCTTGC ATCTGGAACG GGTCTTTCA AATGTGGTAT AGCAGTGGCT CCAGTCTCCA 2160
GCTGGGAATA TTACCGTCT GTCTACACAG AGAGATTCA GGGTCTCCCA ACAAAAGATGA 2220
TAATCTTGAG CACTATAAGA ATTCAACTGT GATGGCAAGA GCAGAATATT TCAGAAATGT 2280
AGACTATCTT CTCATCCACG GAACAGCAGA TGATAATGTG CACTTTCAA ACTCAGCACA 2340
15 GATTGCTAAA GCTCTGGTTA ATGCACAAAGT GGATTTCCAG GCAATGTGGT ACTCTGACCA 2400
GAACCACGGC TTATCCGCC TGTCCACGAA CCACTTATAC ACCCACATGA CCCACTTCCT 2460
AAAGCAGTGT TTCTCTTGT CAGACTAAAA ACGATGCAGA TGCAAGCCTG TATCAGAAATC 2520
TGAAAACCTT ATATAAACCC CTCAGACAGT TTGCTTATT TATTTTTAT GTTGTAAAAT 2580
GCTAGTATAA ACAAACAAAT TAATGTTGTT CAAAGGCTG TTAAAAAAA GATGAGGACT 2640
20 CAGAAGTTCA AGCTAAATAT TGTTTACATT TTCTGGTACT CTGTGAAAGA AGAGAAAAGG 2700
GAGTCATGCA TTTTGCTTG GACACAGTGT TTTATCACCT GTTCATTTGA AGAAAAATAA 2760
TAAAGTCAGA AGTTCAAAAAA AAAAAAAA AAAAAAAA GCGGCCGCTC GA 2812

5 We claim:

1. Isolated nucleic acid molecule which codes for mammalian FAP α having a molecular weight of about 88 kilodaltons based upon its deduced amino acid sequence.
- 10 2. The isolated nucleic acid molecule of claim 1, wherein said FAP α consists of the amino acid sequence set forth in SEQ ID NO: 1.
- 15 3. The isolated nucleic acid molecule of claim 1, consisting of the nucleotide sequence of SEQ ID NO: 1.
- 20 4. Isolated nucleic acid molecule which hybridizes to the nucleotide sequence of SEQ ID NO: 1, under stringent conditions.
- 25 5. Expression vector comprising the isolated nucleic acid molecule of claim 1, operably linked to a promoter.
- 30 6. Cell line transformed or transfected by the isolated nucleic acid molecule of claim 1.
- 35 7. Cell line transformed or transfected by the expression vector of claim 5.
8. Method for determining expression of FAP α in a cell comprising contacting said cell with the isolated nucleic acid molecule of claim 1 and determining hybridization of said isolated nucleic acid molecule to a complementary sequence in said cell as a determination of expression of FAP α .

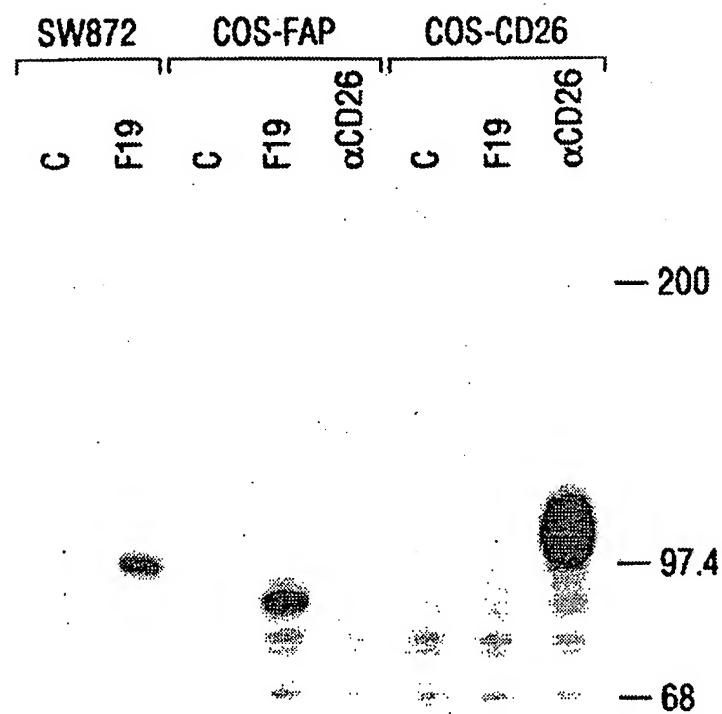
FIG. 1

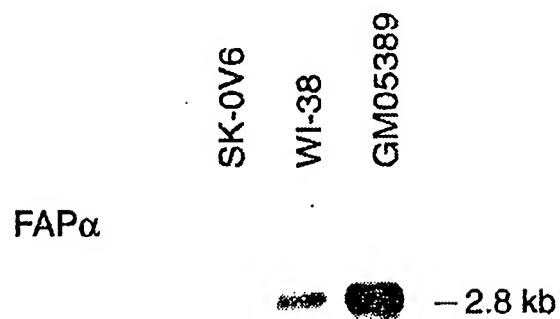
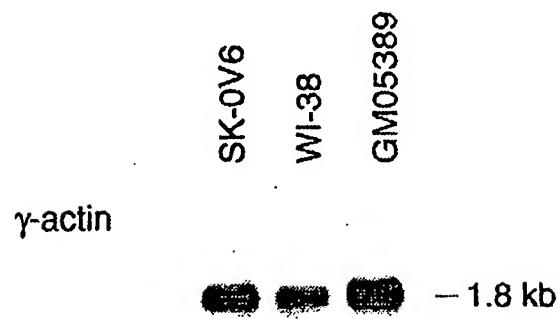
FIG. 2A**FIG. 2B**

FIG. 3

FAP	1	MKTWVKIVFGV*ATSAVLALLVMCIVLRPSRVHNSEENTMRALTLKDILN	49
CD26	1	---PW-VLL-LLGAA-LVTIITVPV--LNKGTDATADSRKTY--T-Y-K	50
FAP	50	GTFSYKTFFPNWISGQEYLHQSAADNNIVLYNIETGQSYTILSNRTMKS*	98
CD26	51	N-YRL-LYSLR---DH---YKQ*E---LVF-A-Y-N-SVF-E-S-FDEFG	99
FAP	99	*NASNYGLSPDRQFVYLESDYSKLWRYSYTATYYIYDLSNGEFVRGNELP	147
CD26	100	HSIND-SI---G--IL--YN-V-Q--H---S-D---NKRQLITEERI-	149
		fap-1	
FAP	148	RPIQYLCWSPVGSKLAYVYQNNIYLKQRPGDPPFQITFNGRENKIFNGIP	197
CD26	150	NNT-WVT----H----WN-D--V-IE-NL-SYR--WT-K-DI-Y---T	199
		fap-2	
FAP	198	DWVYEEEMLPTKYALWWSPNGKFLAYAEFNDKDIPVIAYSYYGDE**QYP	245
CD26	200	-----VFSAYS-----T-----Q---TEV-L-E--F-S--SL---	249
FAP	246	RTINIPYPKAGAKNPVVRIFIIDT***TYPAYVGPQEVPVPAMIASSDYY	292
CD26	250	K-VRV-----V--T-KF-VVN-DSLSSVTNATSIQITA--SMLIG-H-	299
FAP	293	FSWLTWVTDERVCLQWLKRVONVSVLICDFREDWQTWDCPKTQEHIIES	342
CD26	300	LCDV--A-Q--IS----R-I--Y--MD---YD-SSGR-N-LVARQ---M-	349
FAP	343	RTGWAGGFFVSRPVFSYDAISYYKIFSDKDGYKHIHYIKDTVENAIQITS	392
CD26	350	T---V-R-RP-E-H-TL-GN-F---I-NEE--R--C-FQIDKKDCTF--K	399
FAP	393	GKWEAINIFRVTQDSLFYSSNEEEYPGRRNIYRISIGSYPPSKKCVTCH	442
CD26	400	-T---V-G-EAL-S-Y-Y-I---YKGM--G--L-K-QLSD-T*KVT-LS-E	448
FAP	443	LRKERCQYYTASFSDYAKYYALVCYGPPISTLHDGRTDQEIKILEENK	492
CD26	449	-NP-----SV---KE---Q-R-S---L-LY---SSVN-KGLRV--D-S	498
		fap-3	
FAP	493	ELENALKNIQLPKEEIKLEVDEITLWYKMLPPQFDRSKYPLLIQVY	542
CD26	499	A-DKM-Q-V-M-SKKLDFIILN-TKF--Q----H--K-----LD--A	548
FAP	543	GPCSQSVRSVFAVNWIISYLASKEGMVIALVDGRGTAFQGDKLLYAVYRKL	592
CD26	549	-----KADT--RL--AT----T-NIIV-SF----SGY---IMH-IN-R-	598
FAP	593	GVYEVEDQITAVRKFIEMGFIDEKRIAIWGSYEIRFITGPCIWNWSFQM	642
CD26	599	-TF-----E-A-Q-SK---V-N-----GGYVTSVVLGSGSGVGFK	648
FAP	643	WYSSGSSLQLGILRVCLHRE*IHGSPNKDDNLEHYKNSTVMARAEYFRNV	691
CD26	649	CGIAVAPVSRWEYYDSVT-RYM-L-TPE---D--R-----S---N-KQ-	698
FAP	692	DYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMWYSDQNHGLSGLSTN	741
CD26	699	E-----Q---S----DVG-----T-ED--IASSTAH	748
FAP	742	*HLYTHMTHFLKQCFSLSD	
CD26	749	Q-I----S--I-----P	

4 / 5

Fig. 5

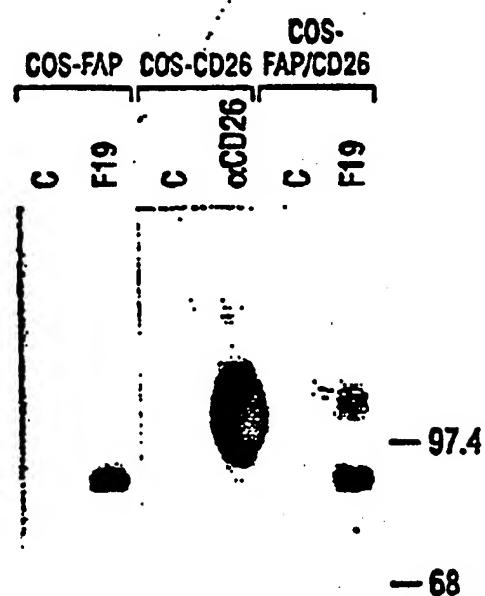
Breast cancer	MFH	Healing wound	Lung cancer
A	C	E	G
B	D	F	H
⊕	⊕	⊕	⊕

FAP α

CD26

Immunohistochemistry (see Korla et al.)

Fig. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04860

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/00, 15/09, 15/12

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 172.3, 240.2, 252.3, 320.1; 536/23.5, 24.3, 24.31, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155,351,357,358) search terms: FAP, fibroblast activation protein, antigen, CD26

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ----	CANCER RESEARCH, Volume 53, issued 15 July 1993, W.J. Rettig et al, "Regulation and Heteromeric Structure of the Fibroblast Activation Protein in Normal and Transformed Cells of Mesenchymal and Neuroectodermal Origin", pages 3327-3335, see entire document.	1-7 ----- 8
Y ----	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, Volume 85, issued May 1988, W.J. Rettig et al, "Cell-surface glycoproteins of human sarcomas: Differential expression in normal and malignant tissues and cultured cells", pages 3110-3114, see entire document.	1-7 ----- 8

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•O" document referring to an oral disclosure, use, exhibition or other means		
•P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

21 JUNE 1995

Date of mailing of the international search report

10 JUL 1995

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04860

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, Volume 84, issued December 1987, A. Aruffo et al, "Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system", pages 8573-8577, see entire document.	1-7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/04860

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/6, 69.1, 172.3, 240.2, 252.3, 320.1; 536/23.5, 24.3, 24.31, 24.33

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These were some of the same SNPs, involving the same high-risk alleles **associated with disease** in the USA and UK families (Jin et al., 2007). **SNP rs8074853 ...**

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SNP rs8074853, located 6.8 kb upstream from **rs6502867**, was marginally **associated with disease** in our previous study (Jin et al., 2007), and was likewise ...

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[\(WO/2002/015048\) METHOD OF DETECTING DISEASE-ASSOCIATED SNP](#)

Namely, a method of detecting **disease-associated SNP(s)** in a gene involving the following steps (a) to (e): (a) the step of grouping a plurality of ...

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SNP analysis system for detecting complex disease associated sites ...

SNP Analysis System for Detecting Complex Disease Associated Sites. Yoko Higashi. 1. , Hirotaka Higuchi. 1. , Takashi Kido. 2. , Hirohito Matsumine ...

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